Kanji ONO* & Ryuso TANAKA**: On the regeneration of setae in the moss *Haplocladium angustifolium*

小野莞爾*・田中隆荘**: 蘚類ノミハニワゴケの蒴柄の再生について

Since the success of the regeneration experiment of setae in moss was reported by Campbell (1905) and Marchal and Marchal (1907), studies on the regenerated moss plants were carried out by several investigators. In the course of his genetic studies in mosses, Wettstein (1923, 1924a, 1924b, 1925, 1928) obtained many kinds of polyploid series by use of the regeneration of setae in *Funaria hygrometrica, Physcomitrium pyriforme,* its intergeneric hybrid, and some other species. Redfearn and Meyer (1949) investigated in *Physcomitrium turbinatum* the effect of position of the regeneration of setae. In these papers anatomical observations of the regenerating cells were not reported.

We found a moss, *Haplocladium angustifolium,* which showed a high rate of regeneration of setae. The purpose of the present investigation is to observe the regeneration of setae in this moss with respect to the relationship between the regeneration and the polarlity of seta segments, the seta tissues from which the protonemata develop, and the cytological features of the development of protonema.

Materials and methods The materials of *Haplocladium angustifolium* (Hampe et C. Muell.) Broth. were collected at Hiroshima-city, Hiroshima Prefecture, and Kitsuki-city, Oita Prefecture. For the experiments of regeneration of setae, relatively young setae of about 10-15 mm long were used. These setae were excised at the basal ends just above the foots and at the upper ends just below the capsules, and the median parts were used for cultures. A modified Burgeff's solution, in which the concentration was ten times higher than in original solution, was used in the present culture. The segments of setae were washed well with sterilized water

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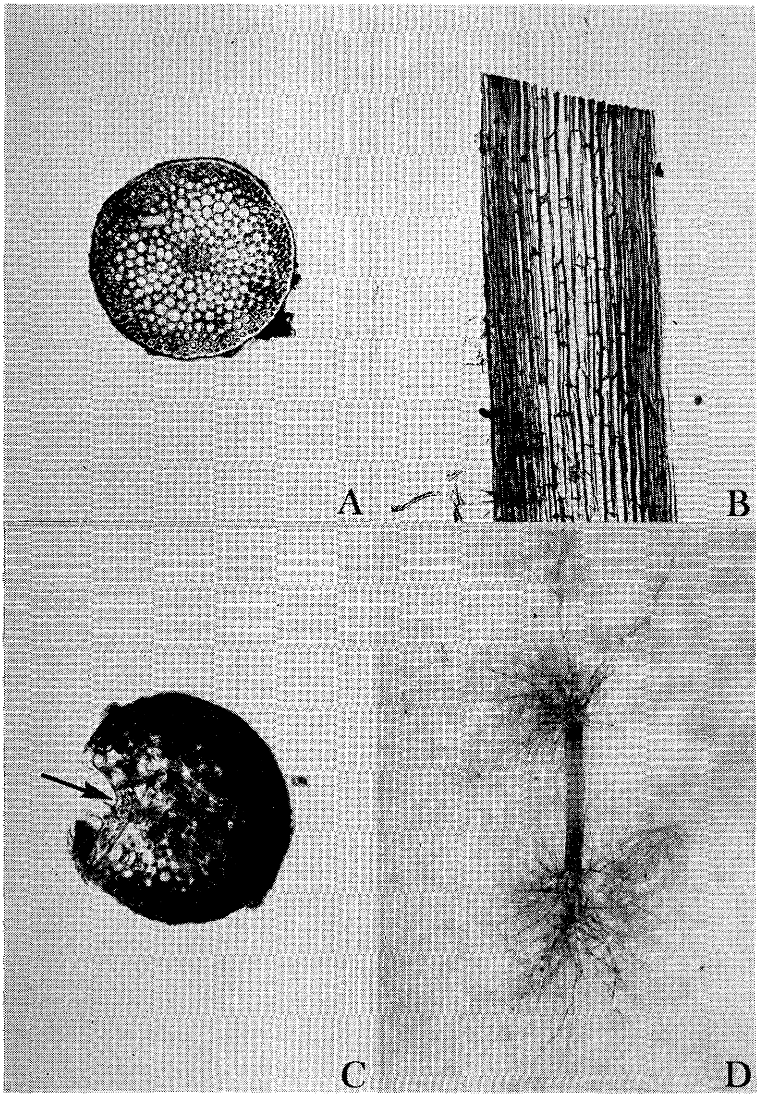

Fig. 1. Regeneration of setae in *Haplocladium angustifolium*. A. Cross section of seta. ×100. B. Longitudinal section of seta. ×100. C. Cross section of regenerating seta (10 days cultures); Arrow shows a protonema developed from tissue. ×100. D. Seta segment regenerated in both ends (35 days cultures). ×10.

and were kept onto filter papers permeated with the nutrient solution in petri dishes. Cultures were maintained at 25°C under fluorescent lighting (200-300 lux).

Results (1) Regeneration rate and polarity in regeneration. After 10 days culture, the protonemata began to develop from the upper ends of seta-segments, but we did not observe protonemata developed from the basal ends of segments. After 20 days culture, however, the development of protonemata from the basal ends was also observed in some of the segments which had produced protonemata from the upper ends (Fig. 1D).

The mode of regeneration of setae (35 days after the start of cultures) is shown in Table 1. Of 133 seta-segments cultured, regeneration occurred in 113 segments (85%). This regeneration rate in *Haplocladium angustifolium* was remarkably high compaired with that of other mosses reported by previous workers. Of 113 segments 42 (31.6%) were regenerated from only the upper ends and the remaining 71 (53.4%) from both upper and basal ends. There was no segment regenerated from only basal ends.

(2) Protonema development. The cross section of the setae of *Haplocladium angustifolium* is shown in Fig. 1A and its longitudinal section in Fig. 1B, respectively; i. e., the two or three peripheral cells were the layers consisted of thick-walled cells; the interior region was composed of thin-walled parenchymatous cells.

When the seta segments were cultured in the nutrient solution, both ends of the segment changed color gradually from green to dark-brown. The protonemata were found to be developed from the interior parenchymatous cells in these dark-brown parts (Figs. 1C, 1D). The mode of the development of protonemata is shown in Fig. 3. At the beginning

Table 1. Regeneration of setae of *Haplocladium angustifolium* (35 days culture)

Position of regeneration in seta segment	No. of seta segment regenerated	Regeneration rate (%)
Both upper and basal ends	71	53.4
Only upper ends	42	31.6
No regeneration	20	15.0
Total	133	100.0

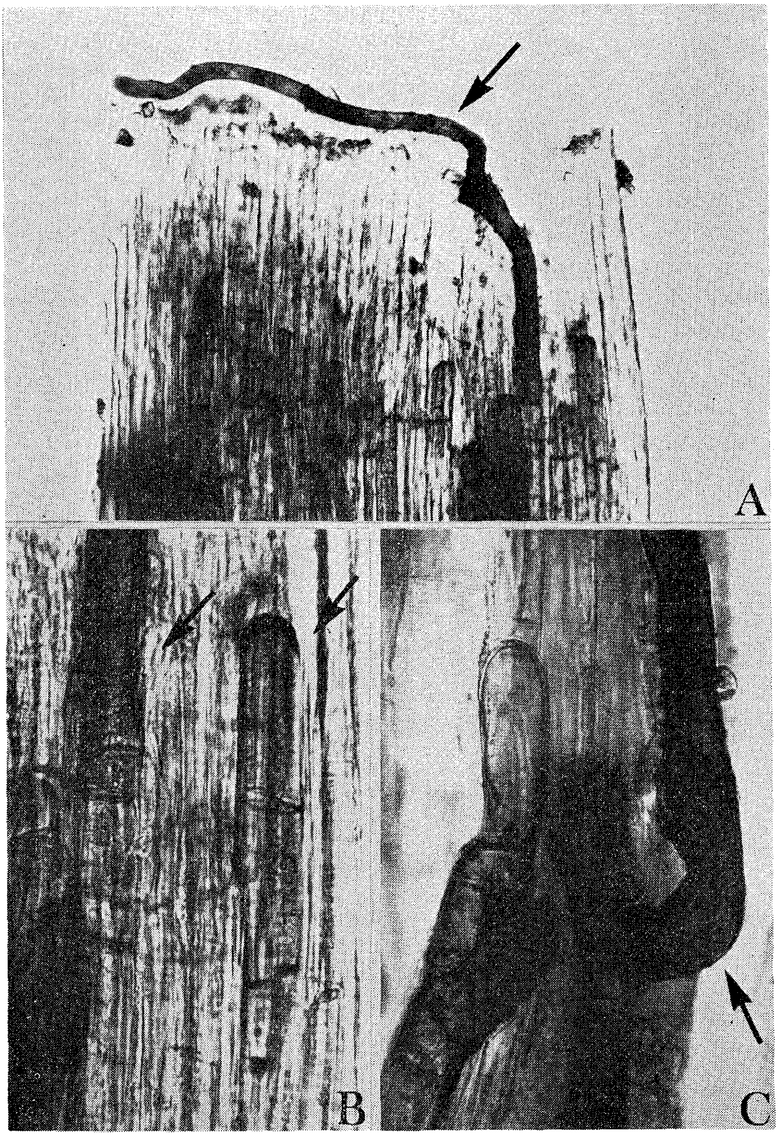

Fig. 2. Regeneration of setae in *Haplocladium angustifolium* (10 days culture). A. Development of protonema from seta (arrow). ×300. B. Protonemata developed from the short side of seta cells (arrows). ×900. C. Protonema developed from the long side of seta cell (arrow). ×900.

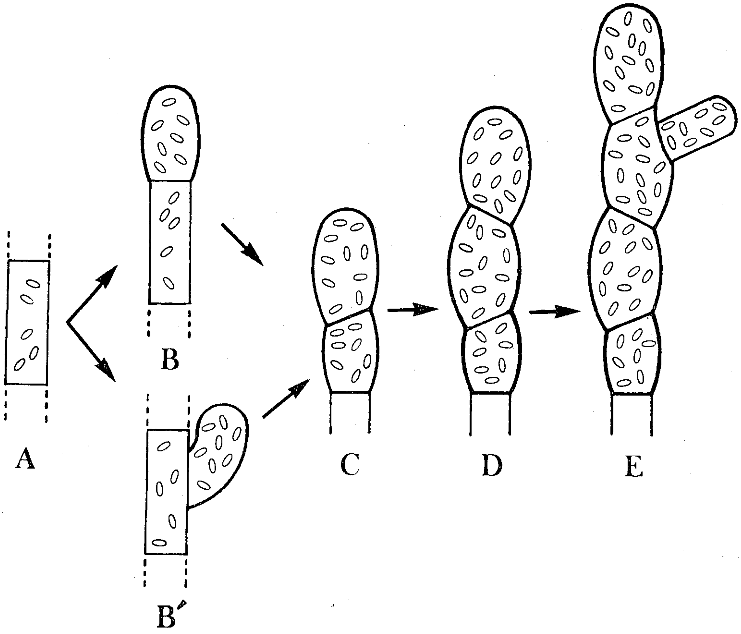

Fig. 3. The process of the regeneration of setae in *Haplocladium angustifolium*.

of the development the parenchymatous cells showed signs of increase in volume. From these increased parenchymatous cells protonemata were formed. The protonemata were found to develop from the apical end of these parenchymatous cells (Figs. 2A, B, 3B). However, it was also found that prototemata developed from the lateral side of the parenchymatous cells (Figs. 2C, 3B′). The protonemata continued an apical growth. The cells of protonemata developed from setae were terete in their shape and connected by slanting septa. They formed many ellipsoidal chloroplasts. These characteristics of cells and chloroplasts were similar to those of caulonemata in the regular protonemata developed from spores.

Discussion Wettstein (1923, 1924, 1925) stated that the regeneration of setae were observed only at the young-upper ends of segments and not at the old-basal ends. On the other hand, Redfearn and Meyer (1949) reported regeneration from besal ends, though it was rare. In the present investigation using *Haplocladium angustifolium*, the protonemata were observed to develop from both upper and basal ends. In this material it was also found that protonemata developed at first from upper ends of seta-segments and subsequently from basal ends. When regeneration of seta was observed in only one end of a segment, it took place always in the upper one. This suggested that the ability of regeneration of seta is related to the age of the tissue.

With regard to the regeneration of leaves in *Brothera leana,* Noguchi and Furuta (1958) mentioned that no regeneration was found in unwounded leaves. The regeneration of *Haplocladium* setae in the present study was also observed in only the parts of setae wounded by cutting. In the regeneration, protonemata were found to develop from the internal-parenchymatous cells of seta tissues. Redfearn and Meyer (1949) reported that protonemata developed also from epidermis of setae in addition to the internal parenchyma, while the authors did not observe such a case.

The authors are grateful to Dr. H. Ando, Hiroshima University, for the identification of the materials studied.

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蘚類 *Haplocladium angustifolium* ノミハニワゴケの蒴柄の再生が，10倍に濃度を高めた Burgeff 培養液で著しく高率で起ることが見出された。蒴柄の再生は，まず蒴柄切片の若い上端部で起こり，続いて古い基部で起こる場合とそうでない場合とがある。基部のみで再生が見られた例はなかった。原糸体は，蒴柄組織の内部の柔組織状細胞から発生し，まずこの細胞の肥大が起こり，次いでこの肥大細胞から分化する。

□台湾植物誌編輯委員会： **Flora of Taiwan, I** （台湾植物誌，第1巻，蕨類・裸子植物）現代関係出版社（台北）1975. A5とB5の中間の判，562ページ，色彩口絵4ページ，英文。US $ 22。中国国家科学委員会と米国 National Science Foundation とによる中・美（すなわち米）科学合作計画に基づいて，台湾の維管束植物のフロラ誌を作る編集委員会が1972年に発足した。委員は5名で，委員長の李恵林と小山鉄夫の両氏が米国側，De Vol・劉棠瑞・黄増泉の3氏が中国側である。それぞれ持ち前の部門を担当，ほかに多数の専門家を動員して事業を進め，昨年6月に原稿が揃ったという。何しろ植物群によって研究の進み方や方法がまちまちで，全体としての統一が非常にむずかしかったと緒言に書いてあるが，これだけの短期間にこのように立派なものを完成させたことは非常な努力であったことと想像される。植物学の研究にはもちろん農学や教育などの方面にも大いに役立つ書物である。

全5巻，それに文献集と総索引の1巻が付属する予定で第1巻はシダ植物と裸子植物である。シダ植物は前記の De Vol 氏が最も多くを受け持ち，それに謝萬權ら数氏がそれぞれ得意の群を分担，日本から大悟法滋氏が参加（イノデ属）している。シダ植物の大分けは普通のやり方どおりマツバラン綱・ヒカゲノカズラ綱・トクサ綱・シダ綱の4綱に，シダ綱は途中の階級を設けずに直ちに23科に分類する。この分類法はコープランド氏などに比べてワラビ科・オシダ科・ウラボシ科などがそれぞれ数科に分かれる近ごろのやり方の一つであるが，中国高等植物図鑑ほどには細分してなく，また科の配列順も違う。多分この方式は独特な今回初めてのものらしく，興味ある分類系のようにみえる。シダ植物は全部で37科・158属・561種（*Grammitis* の新種1を含む）で，属の取扱いは細かい（特にヒメシダ科など）。科・属・種にはそれぞれ記載があり，それらの間には検索表，各種（変種がある場合には各変種）には学名の出典（新組合せ若干あり），主要文献，中国名，異名，分布，台湾内の産地と採集者名など，さらに1ページ大の凸版図が169個あって各属につき1種以上の図を用意している。この図は Taiwania で見たことのあるものも若干あるが，ほとんどが今回描きおろしのもので実によく描けており詳しい部分図をもつている。このような工合でまことに使いやすく，わかりやすいフロラ誌になっている。裸子植物は50ページ，委員長の李恵林氏が担当，8科・16属・25種が収められ，全種について図がある。　（伊藤　洋）